FACULTY OF VETERINARY MEDICINE
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INCIDENCE OF CITRININ IN THE BELGIAN FEED CHAIN AND ITS TOXICOKINETIC PROFILE IN BROILER CHICKENS

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INTRODUCTION

- A scientific opinion on **CITRININ (CIT)** published by **EFSA**[1] emphasized that
  - **additional quantitative occurrence and toxicity data** is needed;
  - the impact of **uncertainties** on the risk assessment is large;
  - more data regarding the toxicity and the occurrence of CIT in food and feed are needed to enable refinement.
- In **Belgium**, a **risk assessment** will be performed in order to set legal limits for this toxin, by using collected occurrence and toxicity data of CIT.

OBJECTIVES

- To collect **occurrence data** of **CIT** in feed in Belgium
  - an LC-MS/MS method was developed for analysis of CIT in feed (pig and chicken);
  - this validated method was applied on 90 Belgian feedstuffs.
- To collect **toxicokinetic data** of **CIT** and its metabolite dihydrocitrinone (**HO-CIT**) in broiler chickens
  - an LC-MS/MS method was developed for analysis of CIT and HO-CIT in chicken plasma;
  - a pilot toxicokinetic study was performed on 1 broiler chicken.

MATERIALS AND METHODS

Collection of samples

FEED

A total of 90 feed samples (broiler chicken and pig feed) were obtained from different Belgian feed producing companies from March 2017 until June 2017.

CHICKEN PLASMA

A dose of 0.25 mg/kg body weight of CIT (in physiological saline) was administered intravenously to 1 broiler chicken (Ross 308, 4 weeks old, ♂). Blood (200 µL, leg vein) was collected at 0, 0.08, 0.16, 0.33, 0.66, 0.75, 1, 2, 3, 4, 6, 24, 48 and 72 h after administration.
The trial was approved by the Ethical Committee of the Faculties of Bioscience Engineering and Veterinary Medicine from Ghent University (case no. EC 2017/105).

Sample pretreatment

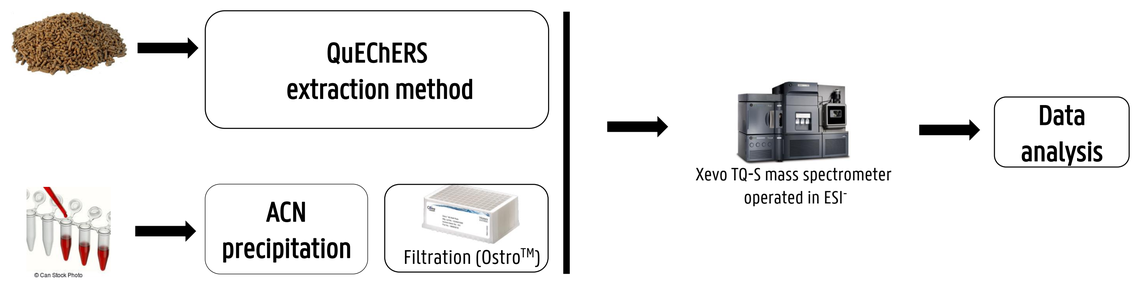

Fig 1: Overview sample preparation for extraction of CIT in feed and chicken plasma

RESULTS

Method validation CIT/HO-CIT in feed and chicken plasma

Table 1: Method performance parameters of the developed LC-MS/MS method for analysis of CIT/HO-CIT in animal feed and chicken plasma.

	FEED	CHICKEN PLASMA	
	CIT	CIT	HO-CIT
Range	1 – 250 µg/kg	0.1 – 100 ng/mL	0.1 – 100 ng/mL
Apparent recovery	80-107%	80-107%	80-110%
RSD_R	<5 %	<20 %	<20 %
LOD	0.5 µg/kg	0.05 ng/mL	0.05 ng/mL
LOQ	1 µg/kg	0.1 ng/mL	0.1 ng/mL

Occurrence CIT in FEED

Table 2: Occurrence of CIT in Belgian chicken and pig feed. Concentrations are shown with respect to their measurement uncertainty. *Of samples above LOQ (1 µg/kg)

Feed	Toxin	% Positive samples	Average concentration* (µg/kg)	Max concentration (µg/kg)
Chicken (n = 38)	CIT	45%	1.95 ± 0.46	3.9 ± 0.92
Pig (n = 52)	CIT	51%	1.58 ± 0.37	3.70 ± 0.87

CIT was detected in about half of the sampled feedstuffs (Table 2).

Preliminary results toxicokinetic profile CIT in chicken plasma

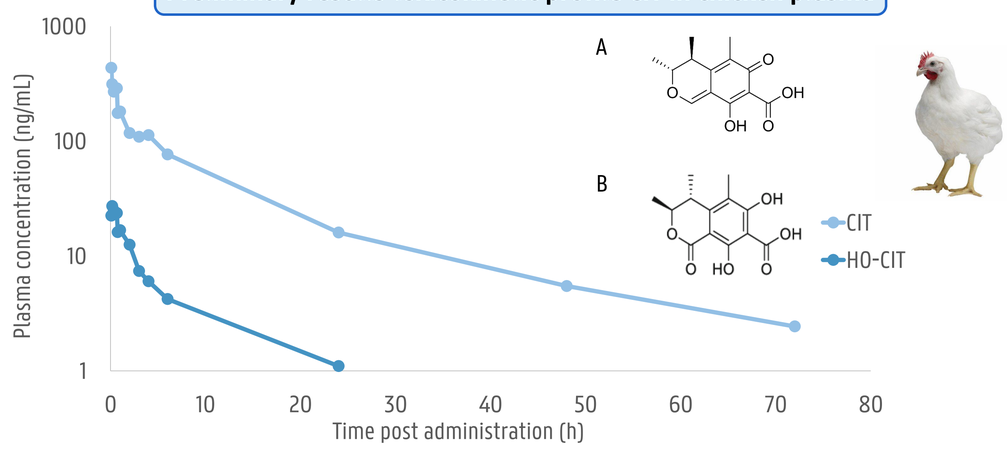

Fig 2. Plasma concentration–time profile of CIT and HO-CIT after single intravenous administration of 0.25 mg CIT /kg body weight to broiler chicken (n=1 ♂). The inserts are the chemical structures of (A) CIT and (B) HO-CIT.

Compared to Devreese et al[1]. (2018), the preliminary elimination rate constant (K_{el}) of CIT in broiler chickens is in line with the K_{el} of **ochratoxin A** after IV administration of 0.25 mg/kg body weight (Table 3). More data from a toxicokinetic study with a sufficient number of animals (n = 8) should confirm this.

Table 3. Comparison of toxicokinetic parameters of CIT and OTA[1] after intravenous administration of 0,25 mg/kg body weight to broiler chickens (CIT: n = 1 ♂/OTA: n = 4 ♀/ 4 ♂),

	C_0 (ng/L)	AUC_{0-72h} (h*ng/L)	K_{el} (1/h)	$T_{1/2el}$ (h)	V_d (L/kg)	Cl (L/h/kg)
OTA	910.52 ± 627.31	335.57 ± 79.30	0.045 ± 0.021	23.95 ± 15.27	19.85 ± 11.74	0.61 ± 0.12
CIT	434.69	1920.80	0.04	17.68	3.32	0.13

CONCLUSIONS AND FURTHER RESEARCH

- ✓ **CIT** frequently **occurs** in Belgian feed, although in low concentration levels
- ✓ Limited data is available concerning its **toxicity** and toxico**kinetics**.
- ✓ Hence, further **research** is needed.

Toxicokinetic study: Toxicokinetic parameters?

Steady-state study: Carry-over to edible tissues? (Fig. 3)

Post-mortem evaluation: Organ damage?

MetID (HRMS): CIT phase I and phase II metabolites

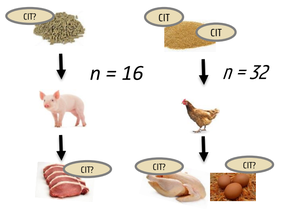

Fig. 3: Presentation of experimental set-up for carry-over study

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[1] M. Devreese, S. Croubels, S. De Baere, R. Gehring, G. Antonissen, Comparative Toxicokinetics and Plasma Protein Binding of Ochratoxin A in Four Avian Species, J. Agric. Food Chem. 66 (2018) 2129–2135..

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